CLAIMS

1. A method for *in vivo* down-regulation of growth differentiation factor 8 (GDF-8) activity in an animal, including a human being, the method comprising effecting presentation to the animal's immune system of an immunogenically effective amount of

- at least one GDF-8 polypeptide or subsequence thereof which has been formulated so that immunization of the animal with the GDF-8 polypeptide or subsequence thereof induces production of antibodies against the GDF-8 polypeptide, and/or
- at least one GDF-8 analogue wherein is introduced at least one modification in the GDF-8 amino acid sequence which has as a result that immunization of the animal with the analogue induces production of antibodies against the GDF-8 polypeptide.

2. The method according to claim 1, wherein is presented a GDF-8 analogue with at least one modification of the GDF-8 amino acid sequence.

3. The method according to claim 2, wherein the modification has as a result that a substantial fraction of GDF-8 B-cell epitopes are preserved and that

- at least one foreign T helper lymphocyte epitope (T_H epitope) is introduced, and/or
- at least one first moiety is introduced which effects targeting of the modified molecule to an antigen presenting cell (APC) or a B-lymphocyte, and/or
- at least one second moiety is introduced which stimulates the immune system, and/or

- at least one third moiety is introduced which optimises presentation of the modified GDF-8 polypeptide to the immune system.

4. The method according to claim 3, wherein the modification includes introduction as side groups, by covalent or non-covalent binding to suitable chemical groups in GDF-8 or a subsequence thereof, of the foreign T_H epitope and/or of the first and/or of the second and/or of the third moiety.

5. The method according to claim 3 or 4, wherein the modification includes amino acid substitution and/or deletion and/or insertion and/or addition.

6. The method according to claim 5, wherein the modification results in the provision of a fusion polypeptide.

7. The method according to ~~claim 5 or 6~~ claim 5, wherein introduction of the amino acid substitution and/or deletion and/or insertion and/or addition results in a substantial preservation of the overall tertiary structure of GDF-8.

8. The method according to ~~any one of claims 2-7~~ claim 2, wherein the modification includes duplication of at least one GDF-8 B-cell epitope and/or introduction of a hapten.

9. The method according to ~~any one of claims 3-8~~ claim 3, wherein the foreign T-cell epitope is immunodominant in the animal.

10. The method according to ~~any one of claims 3-9~~ claim 3, wherein the foreign T-cell epitope is promiscuous, such as a natural promiscuous T-cell epitope and an artificial MHC-II binding peptide sequence.

11. The method according to claim 10, wherein the natural T-cell epitope is selected from a Tetanus toxoid epitope such as P2 or P30, a diphtheria toxoid epitope, an influenza virus hemagluttinin epitope, and a *P. falciparum* CS epitope.

12. The method according to ~~any one of claims 3-11~~ claim 3, wherein the first moiety is a substantially specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen, such as a hapten or a carbohydrate for which there is a receptor on the B-lymphocyte or the APC, such as mannan or mannose.

13. The method according to ~~any one of claims 3-12~~ claim 3, wherein the second moiety is selected from a cytokine, a hormone, and a heat-shock protein.

14. The method according to claim 13, wherein the cytokine is selected from, or is an effective part of, interferon γ (IFN-γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF), and wherein the heat-shock protein is selected from the group consisting of HSP70, HSP90, HSC70, GRP94, and calreticulin (CRT), or an effective part thereof.

15. The method according to ~~any one of claims 3-14~~ claim 3, wherein the third moiety is of lipid nature, such as a palmitoyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group.

16. The method according to ~~any one of the preceding claims~~ claim 1, wherein the GDF-8 subsequence or the GDF-8 analogue is derived from the C-terminal, active form of GDF-8, such as subsequence

or analogue derived from a bovine, porcine, human, chicken, sheep, or turkey GDF-8 polypeptide.

17. The method according to claim 16, wherein the GDF-8 polypeptide has been modified by substituting at least one amino acid sequence in SEQ ID NO: 11 or 12 with at least one amino acid sequence of equal or different length which contains a foreign T_H epitope, wherein substituted amino acid sequences are comprised in residues 1-12, 18-41, 43-48, 49-69, or 79-104 in SEQ ID NO: 11 or 12, or wherein the GDF-8 polypeptide has been modified by inserting at least one amino acid sequence which contains a foreign T_H epitope, wherein insertion is performed anywhere in positions 1-12, 18-30, 42-51, 82-86, and 105-109 in SEQ ID NO: 11 or 12.

18. The method according to ~~any one of the preceding claims~~ claim 1, wherein presentation to the immune system is effected by having at least two copies of the GDF-8 polypeptide, the subsequence thereof or the modified GDF-8 polypeptide covalently of non-covalently linked to a carrier molecule capable of effecting presentation of multiple copies of antigenic determinants.

19. The method according to ~~any one of the preceding claims,~~ claim 1 wherein an effective amount of the GDF-8 polypeptide or the GDF-8 analogue is administered to the animal via a route selected from the parenteral route such as the intradermal, the subdermal, the intracutaneous, the subcutaneous, and the intramuscular routes; the peritoneal route; the oral route; the buccal route; the sublinqual route; the epidural route; the spinal route; the anal route; and the intracranial route.

20. The method according to claim 19, wherein the effective amount is between 0.5 µg and 2,000 µg of the GDF-8 polypeptide, the subsequence thereof or the analogue thereof.

21. The method according to claim 19 or 20, which includes at least one administration of the GDF-8 polypeptide or analogue per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations per year.

22. The method according to ~~any one of claims 19-21~~ claim 19, wherein the GDF-8 polypeptide, the subsequence thereof, or the modified GDF-8 polypeptide optionally has been formulated with a pharmaceutically and immunologically acceptable carrier and/or vehicle and has been formulated with an adjuvant which facilitates breaking of autotolerance to autoantigens, such as an adjuvant selected from the group consisting of an immune targeting adjuvant; an immune modulating adjuvant such as a toxin, a cytokine and a mycobacterial derivative; an oil formulation; a polymer; a micelle forming adjuvant; a saponin; an immunostimulating complex matrix (an ISCOM matrix); a particle; DDA; aluminium adjuvants; DNA adjuvants; γ-inulin; and an encapsulating adjuvant.

23. The method according to ~~any one of claims 20-22~~ claim 20, wherein the GDF-8 polypeptide or analogue is contained in a virtual lymph node (VLN) device.

24. The method according to ~~any one of claims 1-18~~ claim 1, wherein presentation of modified GDF-8 to the immune system is effected by introducing nucleic acid(s) encoding the modified GDF-8 into the animal's cells and thereby obtaining *in vivo* expression by the cells of the nucleic acid(s) introduced.

25. The method according to claim 24, wherein the nucleic acid(s) introduced is/are selected from naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA included in a viral vector, DNA formulated with a

transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA formulated with chitin or chitosan, and DNA formulated with an adjuvant.

26. The method according to claim 24 or 25, wherein the nucleic acids are administered intraarterially, intravenously, or by the routes defined in claim 19.

27. The method according to claim 24 or 25, wherein the nucleic acid(s) is/are contained in a VLN device and/or are formulated as defined in claim 22.

28. The method according to ~~any one of claims 25-27~~ claim 25, which includes at least one administration of the nucleic acids per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations per year

29. A method for increasing the muscle mass of an animal, the method comprising down-regulating GDF-8 activity according to the method of ~~any one of claims 1-28~~ claim 1 to such an extent such that the muscle mass is increased at least 5% when compared to animals which exhibit normal GDF-8 activity, such as at least 10, 15, 20, 25, 30, 35, 40, and 45%.

30. A GDF-8 analogue which is derived from an animal GDF-8 polypeptide wherein is introduced a modification which has as a result that immunization of the animal with the analogue induces production of antibodies against the GDF-8 polypeptide, such as a modification as defined in ~~any one of claims 1-18~~ claim 1.

31. An immunogenic composition comprising an immunogenically effective amount of a GDF-8 polypeptide autologous in an ani-

mal, said GDF-8 polypeptide being formulated together with an immunologically acceptable adjuvant so as to break the animal's autotolerance towards the GDF-8 polypeptide, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle.

32. An immunogenic composition comprising an immunogenically effective amount of an GDF-8 analogue according to claim 29, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle and optionally an adjuvant.

33. An immunogenic composition according to claim 31 or 32, wherein the adjuvant is selected from the group consisting of the adjuvants of claim 22.

34. A nucleic acid fragment which encodes an GDF-8 analogue according to claim 29.

35. A vector carrying the nucleic acid fragment according to claim 34, such as a vector capable of autonomous replication.

36. The vector according to claim 35 which is selected from the group consisting of a plasmid, a phage, a cosmid, a minichromosome, and a virus.

37. The vector according to claim 35 or 36, comprising, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to claim 34, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to claim 34, and optionally a terminator.

65 claim 41

38. The vector according to ~~any one of claims 41-44~~ which, when introduced into a host cell, is either capable of being integrated in the host cell genome or which is not capable of being integrated in the host cell genome.

claim 37

39. The vector according to ~~claim 37 or 38~~, wherein the promoter drives expression in a eukaryotic cell or drives expression in a prokaryotic cell.

claim 35

40. A transformed cell carrying the vector of ~~any one of claims 35-39~~, such as a transformed cell which is capable of replicating the nucleic acid fragment according to claim 34.

41. The transformed cell according to claim 40, which is a micro-organism selected from a bacterium, such as a bacterium of the genus *Escherichia* (preferably *E. coli*), *Bacillus*, *Salmonella*, or *Mycobacterium* (preferably a non-pathogenic *Mycobacterium* cell such as *M. bovis* BCG), a yeast, a protozoan, or a cell derived from a multicellular organism selected from a fungus, an insect cell such as an S_2 or an SF cell, a plant cell, and a mammalian cell.

42. The transformed cell according to claim 40 or 41, which expresses the nucleic acid fragment according to claim 34, such as a transformed cell which secretes or carries on its surface, the GDF-8 analogue according to claim 30.

claim 1

43. The method according to ~~any one of claims 1-18~~, wherein presentation to the immune system is effected by administering a non-pathogenic micro-organism or virus which is carrying a nucleic acid fragment which encodes and expresses the GDF-8 polypeptide or analogue.

44. The method according to claim 43, wherein the virus is a non-virulent pox virus such as a vaccinia virus or wherein the micro-organism is a bacterium such as the bacterium defined in claim 41.

45. The method according to ~~any claim 43 or 44~~ claim 43, wherein the non-pathogenic micro-organism or virus is administered one single time to the animal.

46. A composition for inducing production of antibodies against GDF-8, the composition comprising

- a nucleic acid fragment according to claim 34 or a vector according to ~~any one of claims 35-39~~ claim 35, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant.

47. The composition according to claim 46, wherein the nucleic acid fragment is formulated according to ~~claim 25 or 27~~ claim 25.

48. A stable cell line which carries the vector according to ~~any one of claims 35-39~~ claim 35 and which expresses the nucleic acid fragment according to claim 34, and which optionally secretes or carries the GDF-8 analogue according to claim 30 on its surface.

49. A method for the preparation of the cell according to ~~any one of claims 40-42~~ claim 40, the method comprising transforming a host cell with the nucleic acid fragment according to claim 34 or with the vector according to ~~any one of claims 35-39~~ claim 35.

50. A method for the identification of a modified GDF-8 polypeptide which is capable of inducing antibodies against unmodified GDF-8 in an animal species where the unmodified GDF-8 polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified GDF-8 polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an GDF-8 polypeptide of the animal species thereby giving rise to amino acid sequences in the set which comprise T-cell epitopes which are foreign to the animal species,
- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified GDF-8, and
- isolating the member(s) of the set which significantly induces antibody production against unmodified GDF-8 in the animal species.

51. A method for the preparation of an immunogenic composition comprising at least one modified GDF-8 polypeptide which is capable of inducing antibodies against unmodified GDF-8 in an animal species where the unmodified GDF-8 polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified GDF-8 polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an GDF-8 polypeptide of the animal species thereby giving rise to amino acid sequences in the set comprising T-cell epitopes which are foreign to the animal,
- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified GDF-8, and
- admixing the member(s) of the set which significantly induces production of antibodies in the animal species which are reactive with GDF-8 with a pharmaceutically and

immunologically acceptable carrier and/or vehicle, optionally in combination with at least one pharmaceutically and immunologically acceptable adjuvant.

52. The method according to claim 50 or 51, wherein preparation of the members of the set comprises preparation of mutually distinct nucleic acid sequences, each sequence being a nucleic acid sequence according to claim 34, insertion of the nucleic acid sequences into appropriate expression vectors, transformation of suitable host cells with the vectors, and expression of the nucleic acid sequences, optionally followed by isolation of the expression products.